Br. J. Pharmacol. (1991), **102**, 788–790

SPECIAL REPORT

Pinacidil inhibits the ryanodine-sensitive outward current and glibenclamide antagonizes its action in cells from the rabbit portal vein

Zhiling Xiong, Shunichi Kajioka, [1]Takeshi Sakai, [2]Kenji Kitamura & Hirosi Kuriyama

Department of Pharmacology, Faculty of Medicine, Kyushu University, Fukuoka 812, Japan

Pinacidil, a potassium-channel opener, inhibited the ryanodine-sensitive oscillatory outward potassium current induced by Ca released from an intracellular store. Glibenclamide, a blocker of the ATP-sensitive K-channel, prevented the action of pinacidil, suggesting the presence of an additional site (to K channels) for the vasodilator actions of pinacidil at which glibenclamide can act as an antagonist.

Keywords: Pinacidil; glibenclamide; Ca-dependent K current; oscillatory outward current; Ca release; intracellular Ca store sites; K-channel opener; ATP-sensitive K channel

Introduction Pinacidil is a known K-channel opener which activates ATP-sensitive K-channels in cardiac cells (Fan *et al.*, 1990). In smooth muscle cells, the vasodilator actions of pinacidil are thought to be mediated by the activation of ATP-sensitive K-channels (Standen *et al.*, 1989; Okabe *et al.*, 1990; Fan *et al.*, 1990; Kajioka *et al.*, 1990). These workers demonstrated that glibenclamide, a blocker of the ATP-sensitive K-channel, inhibited K-channel activities induced by various K-channel openers. However, it is not yet clear whether pinacidil and glibenclamide act solely on the K-channel or whether pinacidil activates additional vasodilator mechanisms (Cook *et al.*, 1989). In the present experiments, we describe an action of pinacidil which appears not to be related to K-channel opening.

Methods Single smooth muscle cells were prepared by collagenase treatment from rabbit portal vein. Cells were placed in a physiological salt solution (PSS) at room temperature and the patch-clamp technique was performed with a glass electrode (2–4 MΩ) filled with a high-K solution containing 0.1 mM EGTA. For single channel recording, outside-out membrane patches were obtained with electrodes of resistance 5–10 MΩ. The ionic composition of PSS and the high-K solution were as follows (mM): (PSS): NaCl 135, KCl 6, $CaCl_2$ 2.5, $MgCl_2$ 1.2 and glucose 12; (high-K solution): KCl 140, $MgCl_2$ 5, Na_2ATP 5, EGTA 0.1 and glucose 12. For single-channel current recording, 4 mM EGTA was added to the high-K solution and the concentration of free Ca was kept at 0.3 μM by addition of $CaCl_2$. The pH of the solutions was adjusted to 7.25 ± 0.05 with 10 mM HEPES titrated with Tris. Drugs used were pinacidil (Shionogi Pharmac. Ltd.), glibenclamide (Sigma Chem., St. Louis, MO) and ryanodine (Wako Pure Chem, Osaka).

Results Single smooth muscle cells of the rabbit portal vein at a holding potential of −40 mV produced an oscillatory outward current (Ioo; Ohya *et al.*, 1987; sometimes called a spontaneous transient outward current (STOC), Bolton & Lim, 1989). Superfusion of ryanodine (30 μM; Figure 1a) or caffeine (3 mM; data not shown), both of which are known releasers of Ca from its store site, inhibited Ioo within a few min, suggesting that, in the portal vein, the generation of Ioo is closely related to the release of Ca from an intracellular store (Ohya *et al.*, 1987; 1988; Sakai *et al.*, 1988). A high concentration of glibenclamide (30 μM) inhibited the activity of Ioo, but 1 μM glibenclamide had no action on Ioo (Figure 1b). Single channel current recording showed that 100 μM glibenclamide did not inhibit the large conductance Ca-dependent K-channel, which is reported to be closely related to generation of Ioo (Figure 1c(i); Sakai *et al.*, 1988). This idea is supported by the fact that charybdotoxin, a selective blocker of the large conductance Ca-dependent K channel, blocked Ioo (data not shown). Similarly, application of pinacidil (≤ 100 μM) did not change the activity of the large conductance Ca-dependent K-channel (Figure 1c(ii)). On the other hand, with the whole-cell voltage-clamp configuration, application of pinacidil (≥ 3 μM) rapidly produced an outward current and then inhibited Ioo after a delay of several min (Figure 1d(i)). A higher concentration of pinacidil (30 μM) further inhibited Ioo and after its removal, the outward current induced by pinacidil declined but it took over 10 min after washout for Ioo generation to recover (Figure 1d(ii)). Pinacidil (5 μM) neither produced an outward current nor inhibited Ioo, after pretreatment with 1 μM glibenclamide (Figure 1e(i)). However, 8 min after removal of both drugs, reapplication of pinacidil (5 μM) produced an outward current and inhibited the generation of Ioo. If pinacidil was applied first glibenclamide (1 μM) partly restored Ioo and inhibited the pinacidil-induced outward current (Figure 1e(ii)). Amplitude histograms showed that pinacidil reduced the number of Ioo (calculated from amplitudes larger than 100 pA) to 0.21 times of the control, and simultaneous application of glibenclamide partly restored Ioo generation (0.51 times of the control; Figure 1f). As shown in Figure 1g, the relative amount of current carried as Ioo was reduced by application of pinacidil (≥ 1 μM) dose-dependently, and glibenclamide (1 μM) significantly prevented the inhibitory actions of pinacidil.

Discussion The findings of the present experiments indicate (1) that pinacidil not only activates K-channels (Standen *et al.*, 1989; Kajioka *et al.*, 1990), but also inhibits the spontaneous K current induced by Ca release from intracellular stores and (2) that glibenclamide both prevents the pinacidil-induced inhibition of Ioo and blocks the pinacidil-activated outward current.

Inhibition of Ioo by pinacidil was not due to direct blockade of the Ca-dependent K-channel, since the activity of the K channel was not inhibited by pinacidil. Although the possibility that pinacidil might synthesize an endogenous channel-blocker for the Ca-dependent K-channels cannot be excluded, a more plausible explanation for the inhibition of Ioo is that pinacidil inhibits some step involved in the Ca release mechanism and/or the Ca refilling mechanism. Since both ryanodine-sensitive and IP_3-sensitive Ca release channels could modify the generation of Ioo (Ohya *et al.*, 1987; 1988; Sakai *et al.*, 1988), it is difficult to decide whether one or both

[1]Present address: Department of Prosthetic Dentistry, Kyushu Dental College, Kitakyushu, Japan.
[2]Author for correspondence.

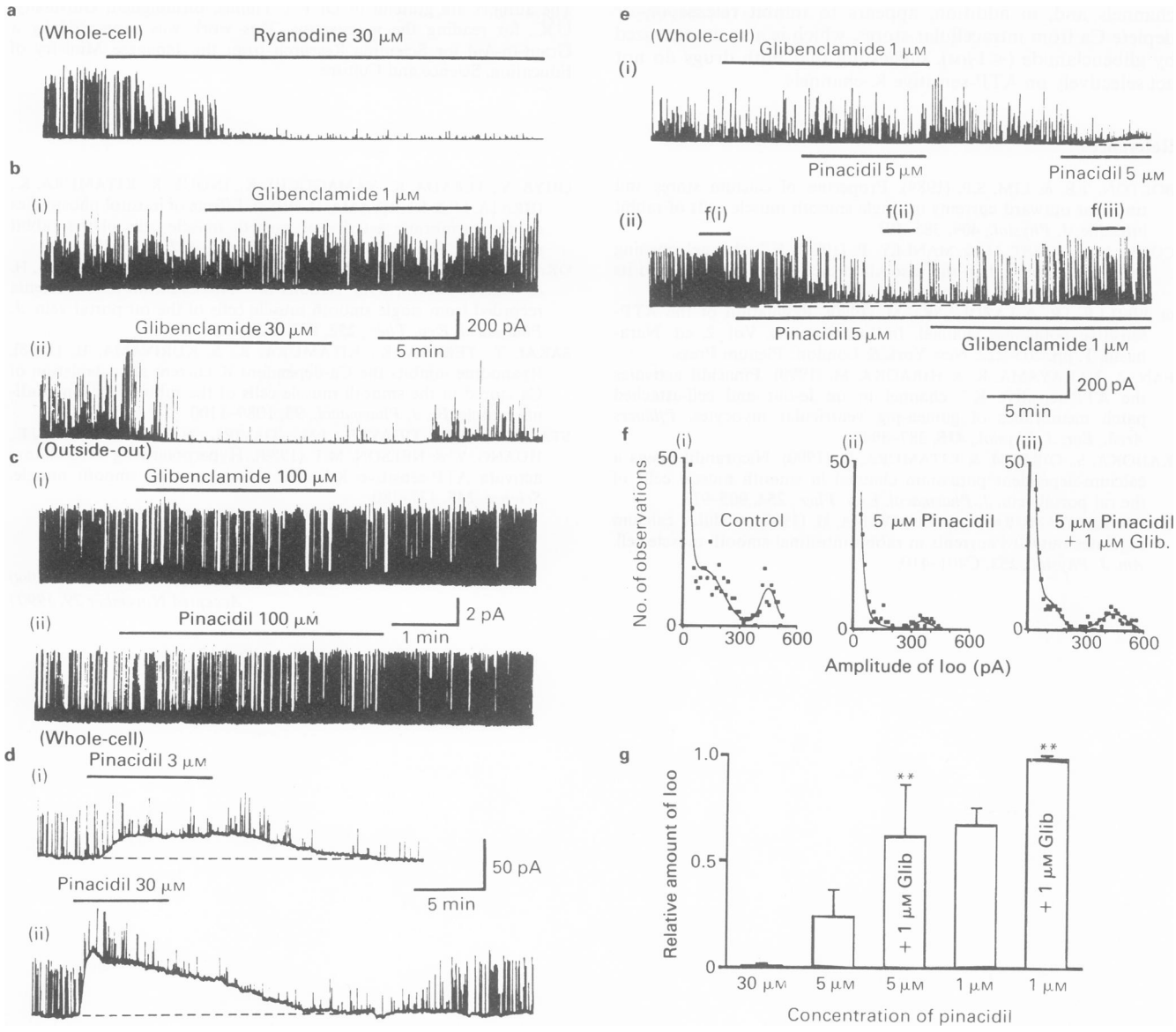

Figure 1 Effects of ryanodine, pinacidil and glibenclamide on the oscillatory outward current (Ioo) and single channel activity of the Ca-dependent K-channel with large conductance. (a) Effect of ryanodine (30 μM) on Ioo. (b) Effect of glibenclamide (1 μM and 30 μM) on Ioo. (c) Effects of glibenclamide (100 μM) and pinacidil (100 μM) on activity of the Ca-dependent K-channel. (d) Effects of pinacidil (3 μM or 30 μM) on Ioo. (e) Antagonism by glibenclamide (1 μM) of the pinacidil (5 μM)-induced inhibition of Ioo. The holding potentials were −30 mV (a, b and e), −40 mV (d) and 0 mV (c). Traces in (c) were recorded with the outside-out membrane patch configuration whereas the other traces were recorded with the whole-cell voltage-clamp configuration. All drugs, dissolved in deionized water and diluted with PSS, were superfused in the bath with a flow rate of 1 ml min^{-1}. (f) Amplitude histograms of Ioo measured in the absence (i) of and presence of 5 μM pinacidil (ii) or in the presence of 5 μM pinacidil and 1 μM glibenclamide (iii). Histograms were obtained from the result shown in Figure 1e(ii) (indicated as f(i), f(ii) and f(iii) above the trace) and the fluctuations of the current less than 30 pA were omitted from the measurement. Curves were fitted by an equation of:

$$Y = A_1 * \mathrm{Exp}(-X/M_1) + A_2 * \mathrm{Exp}(-(X - M_2)^2/S_2^2) + A_3 * \mathrm{Exp}(-(X - M_3)^2/S_3^2)$$

with following values; control, $A_1 = 150$, $M_1 = 20$ pA, $A_2 = 16$, $M_2 = 119$ pA, $S_2 = 130$, $A_3 = 11$, $M_3 = 448$ pA, $S_3 = 58$, correlation coefficient (r) = 0.92; 5 μM pinacidil, $A_1 = 150$, $M_1 = 23$ pA, $A_2 = 1$, $M_2 = 100$ pA, $S_2 = 96$, $A_3 = 2.2$, $M_3 = 353$ pA, $S_3 = 66$, r = 0.96; 5 μM pinacidil + 1 μM glibenclamide, $A_1 = 150$, $M_1 = 25$ pA, $A_2 = 7$, $M_2 = 119$ pA, $S_2 = 70$, $A_3 = 4.9$, $M_3 = 442$ pA, $S_3 = 98$, r = 0.98. (g) Inhibitory actions of pinacidil (1, 5 and 30 μM) on relative amount of current carried as Ioo observed in the absence and presence of 1 μM glibenclamide. Amount of Ioo was estimated by area of amplitude histogram and the histogram in the absence of drug was normalized as 1.0. Each column shows mean value with s.d. ($n = 3$). ** indicates $P < 0.01$ (P-values were calculated between the values in the presence and absence of 1 μM glibenclamide).

processes is responsible for the pinacidil-induced inhibitory action seen in the present experiments.

Glibenclamide is used to inhibit selectively ATP-sensitive K-channel in cardiac muscle and pancreatic β-cells (see review by De Weille & Lazdunski, 1990). In the present experiments, a low concentration of glibenclamide (1 μM) restored the generation of Ioo blocked by pinacidil without any action on Ioo by itself, while a high concentration of the drug (30 μM) had a similar action to pinacidil. This implies that glibenclamide (1 μM) itself, while not having the ability to activate Ioo, did antagonize the inhibitory actions of pinacidil. Although glibenclamide inhibited the pinacidil-induced outward current (ATP-sensitive K current), the antagonizing action of glibenclamide on Ioo was neither related to activation of ATP-sensitive K-channels by pinacidil, as lemakalim had no inhibitory action on Ioo in the same preparation (unpublished observations), nor was it due to direct activation of Ca-dependent K-channels, as glibenclamide had no excitatory action on these channels.

In summary, pinacidil activates glibenclamide-sensitive K-

channels and, in addition, appears to inhibit release of, or deplete Ca from intracellular stores, which is also antagonized by glibenclamide ($\leqslant 1\ \mu M$), suggesting that both drugs do not act selectively on ATP-sensitive K-channels.

The authors are grateful to Dr R.J. Timms, Birmingham University, U.K., for reading the manuscript. This work was supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science and Culture.

References

BOLTON, T.B. & LIM, S.P. (1989). Properties of calcium stores and transient outward currents in single smooth muscle cells of rabbit intestine. *J. Physiol.*, **409**, 385–401.

COOK, N.S., QUAST, U. & MANLEY, P. (1989). K^+ channel opening does not alone explain the vasodilator activity of pinacidil and its enantiomers. *Br. J. Pharmacol.*, **96**, 181P.

DE WEILLE, J.R. & LAZDUNSKI, M. (1990). Regulation of the ATP-sensitive potassium channel. In *Ion Channels*, Vol. 2, ed. Narahashi, T. pp. 205–222. New York & London: Plenum Press.

FAN, Z., NAKAYAMA, K. & HIRAOKA, M. (1990). Pinacidil activates the ATP-sensitive K^+ channel in inside-out and cell-attached patch membranes of guinea-pig ventricular myocytes. *Pflügers Arch., Eur. J. Physiol.*, **415**, 387–394.

KAJIOKA, S., OIKE, M. & KITAMURA, K. (1990). Nicorandil opens a calcium-dependent potassium channel in smooth muscle cells of the rat portal vein. *J. Pharmacol. Exp. Ther.*, **254**, 905–913.

OHYA, T., KITAMURA, K. & KURIYAMA, H. (1987). Cellular calcium regulates outward currents in rabbit intestinal smooth muscle cell. *Am. J. Physiol.*, **252**, C401–410.

OHYA, Y., TERADA, K., YAMAGUCHI, K., INOUE, R., KITAMURA, K., HIRATA, M. & KURIYAMA, H. (1988). Effects of inositol phosphates on the membrane activity of smooth muscle cells of the rabbit portal vein. *Pflügers Arch., Eur. J. Physiol.*, **412**, 382–389.

OKABE, K., KAJIOKA, S., NAKAO, K., KITAMURA, K., KURIYAMA, H. & WESTON, A.H. (1990). Actions of cromakalim on ionic currents recorded from single smooth muscle cells of the rat portal vein. *J. Pharmacol. Exp. Ther.*, **252**, 832–839.

SAKAI, T., TERADA, K., KITAMURA, K. & KURIYAMA, H. (1988). Ryanodine inhibits the Ca-dependent K current after depletion of Ca stored in the smooth muscle cells of the rabbit ileal longitudinal muscle. *Br. J. Pharmacol.*, **95**, 1089–1100.

STANDEN, N.B., QUAYLE, J.M., DAVIES, N.W., BRAYDEN, J.E., HUANG, Y. & NELSON, M.T. (1989). Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. *Science*, **245**, 177–180.

(Received November 19, 1990
Accepted November 29, 1990)